

PROGRESS IN HIGH DENSITY GRIDDED ARRAY HYBRIDIZATION

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Researchers at LLNL and throughout the world continue to make use of high density gridded arrays of transformed bacteria or DNA to support sequencing and gene finding efforts. We are using a commercial robotic positioning system to supplement our Hewlett-Packard ORCA robot, which we have used previously for producing gridded arrays. The new system is capable of producing usable colony arrays at densities approaching 100,000 spots on an 8-cm x 12-cm substrate. We have also developed a 384-pin tool using pins from the semiconductor industry which is more accurate than our previous 384-pin tool. The system is currently being used in a production mode to make arrays at a 6 x 6 x 384 density using this 384-pin tool. These lower-density arrays are suitable for analysis using radio-labeled probes and storage phosphor data capture. Our immediate target is 10 x 10 x 384 arrays of BAC clones analyzed with fluorescently-labeled probes. The new spotting robot has a work envelope of 0.25m x 1m x 2m and allows us to produce up to 164 8-cm x 12-cm filters at a time.

Work is also in progress on a computer aided image analysis system which is interfaced to our genome data base to provide both direct image recovery and automated recording of results. Toward the goal of using fluorescent-based detection methods in very high density array contexts, we are investigating new visible and infra-red labeling strategies and the use of alternative spotting substrates with lower fluorescence backgrounds and improved target presentation. We also describe improvements made to the colony growth, lysis and fixing protocols, including fabrication of a fixture that allows more efficient batch processing of colony filters.

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